Section II (Remarks)

A. Summary of Amendment to the Claims

By the present Amendment, claim 1 has been amended; claims 2 and 3 have been cancelled; and claims 14-19 were previously cancelled. No new matter within the meaning of 35 U.S.C. §132(a) has been introduced by the foregoing amendments. Specifically, the amendment to claim 1 is supported by claim 3, as originally filed.

The amendments made herein are fully consistent with and supported by the originally-filed disclosure of this application.

B. Claim Rejections Under 35 U.S.C. §103

In the Office Action mailed March 4, 2009 the examiner rejected claims 1-5, 7, 11, and 12 under 35 U.S.C. §103(a) as obvious over Francisco, et al., *Proc. Natl. Acad. Sci. USA*, 90 10444-10448, 1993 (hereinafter "Francisco et al.") or Charbit et al., *Gene*, 70, 1, 181-189, 1988 (hereinafter "Charbit et al.") in view of Lee et al, *Trends in Biotechnol.*, 21, 1, 45-52 (hereinafter "Lee et al.") and Christalli et al., *Arch. Biochem. Biophys.* 377, 2, 324-333, 2000 (hereinafter "Christalli et al.").

Additionally the examiner rejected claims 1-7, 11, and 12 under 35 U.S.C. §103(a) as obvious over Francisco, et al. or Charbit et al. in view of Lee and Christalli and further in view of Park et al., *FEMS Microbiol. Lett.* 214, 217, 2002 (hereinafter "Park et al.") or DeBoer et al, *Proc. Natl. Acad. Sci,* USA 80, 21-25, 1983 (Hereinafter "DeBoer et al.").

The examiner also rejected claims 1-5, and 7-12 under 35 U.S.C. §103(a) as obvious over Francisco, et al. or Charbit et al. in view of Lee and Christalli and further in view of U.S. Patent No. 5,508,192 (hereinafter "Georgiou et al.").

Furthermore the examiner rejected claims 1-5, 7, 11, 12, and 13 under 35 U.S.C. §103(a) as obvious over Francisco, et al. or Charbit et al. in view of Lee and Christalli and further in view of U.S. Patent No. 6,071,725 (hereinafter "Pan et al.").

Applicants respectfully traverse all four of the above rejections. All of the rejections are based on the primary references Francisco, et al. or Charbit et al. Additionally, all of the rejections

further rely on secondary references Lee et al. and Christalli et al. The discussion of these four references, as provided below is applicable to all of the stated rejections.

The examiner cited Francisco et al. and Charbit et al. as each "disclos[ing] a vector for expressing a target protein on the surface of cells…" (Office Action mailed March 4, 2009, p. 2.) Lee et al. and Christalli et al. were further cited as "disclos[ing] the outer member protein FadL and its structure…" (Christalli et al.) and "disclos[ing] the general method of displaying proteins of choice on the surface of microbes using fusions such as C-terminal, N-terminal or sandwich type fusions of the protein of choice with 'carrier proteins', which are outer membrane proteins…" (Office Action mailed March 4, 2009, p. 3.)

It is well established that a showing of obviousness must include a reasonable expectation of success. ("The prior art can be modified or combined to reject claims as *prima facie* obvious as long as there is a reasonable expectation of success. *In re Merck & Co., Inc.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986)" MPEP §2143.02) However, one of skill in the art would not have had a reasonable expectation of success in the combination of Francisco et al. or Charbit et al. with Lee et al. and Christalli et al.

The examiner's attention is respectfully drawn to the "Background Art" section of the application, where all four of Francisco, et al., Charbit et al., Lee et al. and Christalli et al. are discussed and the advantages of Applicants' invention are discussed with respect to those references.

A surface-expression system was first developed in the 1980s by expressing peptides or small proteins fused with pIII of a filamentous phage with a relative simple surface. Although the cell surface display using the phage was used in the screening of antibodies, epitopes, high-affinity ligands and the like, the size of proteins which could be expressed on the phage surface was relatively limited. Thus, as an alternative, a cell surface expression method for stably expressing foreign proteins on the surface of microorganisms using a surface protein of microorganisms, such as bacteria or yeasts, as a surface anchoring motif, was developed.

As reported in the 1993 Georgiou reference:

> "[i]n order to express a foreign protein on the surface of cells using the outer membrane protein of a certain organism, a suitable surface protein and the foreign protein should be linked with each other at a gene level to biosynthesize

> a fusion protein, which should be stably passed through a cell inner membrane and attached, and then maintained on the cell surface. For this purpose, a protein having the following properties is preferably selected for use as a surface anchoring motif. Namely, (1) it has a secretion signal capable of passing through the cell inner membrane, at the N-terminal end; (2) it must have a targeting signal which can be stably attached on the surface of a cell outer membrane; (3) it can be expressed on the cell surface in large amounts within range of having no adverse effect on the growth of cells, such that the protein can show high activity; and (4) it is stably expressed regardless of its size such that it should be able to be used in various reactions (Georgiou et al., *Trend. Biotechnol.*, 11 :6, 1993)." (Specification, p. 2; emphasis added)

In view of these desired characteristics, extensive experimentation was still required to arrive at Applicants' claimed invention.

As evidenced by a 2002 article by Chen and Georgiou (included by Supplemental IDS herewith), at that time it was known:

> "[t]hat the display of proteins on the surface of Gram-negative bacteria requires a mechanism by which a desired, recombinant polypeptide: (1) is exported from the cytoplasm, a process typically involving the participation of the protein secretory apparatus of the cell, (2) is targeted to the outer membrane, and (3) can transverse the outer membrane so that it anchors to the external surface…."

But Chen and Georgiou continue, providing:

> "[u]nfortunately, the mechanisms that dictate targeting and insertion of proteins within the outer membrane are not well understood. Moreover, the incorporation of aberrant proteins within the outer membrane can be toxic to the cell." (Emphasis added.)

While various *E. coli* outer membrane proteins such as LamB, OmpA, OmpC, OmpS, and FhuA, etc. are disclosed in Chen and Georgiou (p. 497, 1st col., 1st para.) as available surface anchoring motifs, FadL is not disclosed as an example of surface anchoring motif.

In considering a reference for its effect on patentability, the reference is required to be considered in its entirety, including portions of teach away from the invention under consideration. Simply stated, the prior art must be considered as a whole. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984); MPEP § 2141.02.

Based on the above, it is seen that as of 2002 (as of the time of publication of Chen and Georgiou), some desired characteristics of surface anchoring motifs were known, but the

mechanism was not fully understood and that additional such motifs still remained to be discovered. Applicants performed experimentation in order to result in the discovery of FadL as an effective surface anchoring motif.

Lee et al, *Biotech. and Bioeng.*, 90:223, 2005 (also included by Supplemental IDS herewith; hereinafter "Lee et al. 2005") describes a process for a display of lipase on the cell surface of *E. coli* using OprF as an anchor. As described in the RESULTS and DISCUSSION sections (right column of page 226 to page 229), the fusion site for cell surface display in OprF determines host cell growth. In the 2nd paragraph of the DISCUSSION section, it is described that the authors developed a cell surface display system using the P. *aeruginosa* outer membrane protein OprF as an anchoring motif via C-terminal deletion-fusion strategy and this strategy allowed successful display of lipase in an active form on the surface of *E. coli*. In addition, the inventors described how Val188, Ala196, and Arg213 of OprF were suggested to be possible fusion sites for the display of small peptides, however, Ala196 and Arg 213 were found to be not suitable for larger protein display because cells did not grow well after over-expression of the respective fusion proteins.

Based on the above, it is clear that location of a proper fusion site for target protein display on a cell surface is essential for a success of cell surface display. Discovery of such fusion sites requires extensive experimentation, as expended by applicants, in order to discover the presently claimed invention.

Such experimentation carried out by applicants is apparent in Examples 8-10, where Example 8 demonstrates pH stability of lipase expressed on cell surface and the experimental results showed that more than 90% of the initial activity was maintained even after 48 hours; Example 9 demonstrates temperature stability of lipase expressed on cell surface and the experimental results showed that more than 90% of the activity was maintained even after 120 hours at 50°C; and Example 10 demonstrates maintenance of such activity in organic solvent.

Either of Francisco, et al. or Charbit et al. further in view of Lee et al. and Christalli et al. fails to provide any derivative basis for the claimed invention. In particular, the cited documents fail to demonstrate that all outer membranes are readily functional as a surface anchoring motif. In fact, as evidenced by Lee et al. 2005, provided above, where various fusion positions were found to be unsuitable for larger protein display, it is clear that the position at which a target protein and an

outer membrane protein are fused determines whether target protein has activity or not. Accordingly, one of skill in the art would not have found the claimed vector obvious, in view of the cited references.

None of the additionally cited references: Park et al., DeBoer et al., Georgiou et al., or Pan et al. remedy the deficiencies of the combination of either of Francisco, et al. or Charbit et al. further in view of Lee et al. and Christalli et al.

Based on the foregoing, either of Francisco, et al. or Charbit et al. in view of Lee et al. and Christalli et al. and further in view of any of Park et al., DeBoer et al., Georgiou et al., and Pan et al. fails to provide any logical basis for the vectors, microorganisms or methods recited in claims 1 and 4-13. None of the cited combinations of references renders the claimed invention obvious. Accordingly, withdrawal of the rejections of the claims under 35 U.S.C. § 103(a) as being obvious is respectfully requested.

CONCLUSION

Based on the foregoing, all of applicants' pending claims 1 and 4-13 are patentably distinguished over the art, and in form and condition for allowance. The examiner is requested to favorably consider the foregoing and to responsively issue a Notice of Allowance.

The time for responding to the March 4, 2009 Office Action without extension was set at three months, or June 4, 2009. Applicants hereby request a three month extension of time under 37 CFR § 1.136 to extend the deadline for response to and including September 4, 2009. Payment of the extension fee of $555.00 specified in 37 C.F.R. § 1.17(a)(3), as applicable to small entity, is being made by on-line credit card authorization at the time of EFS submission of this Response. Should any additional fees be required or an overpayment of fees made, please debit or credit our Deposit Account No. 08-3284, as necessary.

If any issues require further resolution, the examiner is requested to contact the undersigned attorneys at (919) 419-9350 to discuss same.

Respectfully submitted,

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